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(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING CCR5 ANTAGONISTS

(57) Abstract: CCR5 antagonists such as anti-CCR5 antibodies, modified chemokines or a fraction thereof, peptides derived from such chemokines, and small organic molecules, are useful for reducing liver inflammation and liver damage caused by HCV infection.

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PHARMACEUTICAL COMPOSITIONS COMPRISING CCR5 ANTAGONISTS

FIELD OF THE INVENTION

The present invention relates to viral infections and, more particularly, to chemokine receptor 5 protein (CCR5) antagonists and their use in the treatment of hepatitis C virus (HCV) infections for reducing liver inflammation and liver damage caused by HCV infection.

BACKGROUND OF THE INVENTION

Chemokines are master regulators of immune cells and hematopoietic stem cells trafficking in the body (Rossi and Zlotnik, 2000). By interaction with seven transmembrane G protein-coupled receptors (chemokine receptors), chemokines mediate the recruitment of leukocytes to sites of inflammation (Rossi and Zlotnik, 2000; Yoshie et al., 2001).

The CC type of chemokine receptor interacts with various signaling proteins, including the monocyte chemoattractant proteins MCP-1, -2, -3, -4, and -5; eotaxin-1; macrophage inflammatory proteins MIP-1α, and MIP-1β; and those regulated upon activation which are normal T-cell expressed and secreted, RANTES. The CCR5 type of chemokine receptor in particular is known to interact with MIP-1α, MIP-1β, and RANTES in monocytes, activated T cells, dendritic cells, and natural killer cells. These β-chemokines do not act on neutrophils but rather attract monocytes, eosinophils, basophils, and lymphocytes with varying degrees of selectivity.

The Th1-associated chemokine receptors CCR5 and CXCR3 and their ligands were suggested to participate in the establishment of hepatic inflammation in hepatitis C virus (HCV) infection (Heydtmann et al., 2001; Shields et al, 1999; Apolinario et al., 2002). In addition, the chemokines CCL21 and CXCL12,

traditionally considered as homeostatic chemokines, further contribute to the establishment of chronic liver inflammation in various chronic liver diseases (Terada et al., 2003; Grant et al., 2002; Goddard et al., 2001).

Hepatitis C is a disease of the liver caused by the HCV virus. The HCC virus invades healthy liver cells, takes over their function and allows the viruses to replicate themselves. In HCV, the inflammatory process is initiated by the host immune system in an attempt to clear the virus (Chang, 2003). Over time, as disease progresses, inflammation mainly contributes to necroinflammatory liver damage, and the healthy liver cells that have been infected will die leaving behind scare tissue, known as fibrosis. Fibrosis will eventually turn into cirrhosis, if the hepatitis C is left untreated - about 20% of HCV chronically infected patients develop liver cirrhosis and 4% of patients develop hepatocellular carcinoma (Seeff, 2002).

The involvement of specific chemokines and their receptors in viral clearance and in the outcomes of chronic HCV infection are yet unknown.

The CCR5 chemokine receptor has been identified as co-receptor for human immunodeficiency virus type 1 (HIV-1) entry in cells. HIV-1 takes advantage of the presence of the chemokine receptor CCR5 to gain access to the cell via a fusion-mediated event. CCR5Δ32, a 32-base pair deletion of the CCR5 gene, is associated with slowed HIV disease progression in heterozygotes and protection against infection in homozygotes.

The CCR5/CCR5Δ32 genotype may be used for assessing the role of CCR5 in HCV infection. It has already been shown that CD3 positive T cells derived from CCR5Δ32 heterozygotes show reduced surface expression of CCR5 and a lower response to its ligands (Wu et al., 1997; Venkatesan et al., 2002). Furthermore, HIV-infected CCR5Δ32 heterozygotes have a delayed disease progression (Paxton et al., 1998; Huang et al., 1996). In accordance with these data, Promrat et al. have suggested that the expression of CCR5 and its ligand RANTES (a beta chemokine that inhibits infection of T cells by primary (M-tropic) HIV-1 strains) may play a role in the modulation of hepatic inflammation (Promrat et al., 2003). Woitas et al. have reported that the frequency of CCR5Δ32 homozygotes was 3-fold higher in a

population of HCV positive patients and concluded that CCR5Δ32 has an adverse host effect in HCV infection (Woitas et al., 2002). In contrast, Promrat et al. (2003) have not found a correlation between CCR5Δ32 allele frequency and susceptibility to HCV infection. This contradicting finding may be explained by the fact that in the study of Woitas et al. (2002), all HCV infected patients were HIV seronegative hemophiliacs, and this could have resulted in the pre-selection of CCR5Δ32 homozygotes in the study group (Zhang et al., 2003; Mangia et al., 2003).

Hepatitis C virus (HCV) is now known to cause most cases of what was previously termed non-A,non-B (NANB) hepatitis. HCV is a single-stranded RNA virus of a type called a flavivirus and causes the vast majority of posttransfusion and sporadic NANB hepatitis. Most cases of hepatitis C are subclinical, even in the acute stage. The infection has a much higher rate of chronicity (about 75%) than hepatitis B. Therefore, hepatitis C is often uncovered by the serendipitous detection of anti-HCV in apparently healthy persons.

There are genetically different variants of HCV, called genotypes, with varying amino acid sequences. These genotype families, at least six (genotypes 1-6), have numerous subtypes. A patient usually is infected with only one subtype, but each subtype is actually a mixture of closely related viruses called quasispecies. These quasispecies have the ability to mutate during treatment and become resistant to interferon therapy. Genotype 1b is one of the most common strains of hepatitis C in the United States and is also the most difficult to treat. Mutation of the virus is suspected as the main reason that most patients fail to clear the virus on their own. The HCV virus's ability to mutate is also the main reason the virus is so hard to eradicate. Viruses that are not destroyed during treatment can quickly mutate and create resistant strains. This propensity hampers vaccine development and, in fact, there is no vaccine against HCV.

Current therapy for chronic hepatitis C includes treatment with interferon-α, (IFN-α), that has to be injected subcutaneously at least three times weekly, optionally in combination with oral ribavirin, that is administered daily. The

treatment is expensive and produces several bothersome side effects. It would be highly desirable to develop additional non-vaccine therapies for hepatitis C.

SUMMARY OF THE INVENTION

It has now been found, in accordance with the present invention, that the CCR5Δ32 allele does not alter the susceptibility of an individual to HCV infection. It has been further found that decreased levels of CCR5 reduce liver inflammation and liver damage in early stages of the disease. These findings mark CCR5 as a target for novel immune modulating drugs for the treatment of HCV.

The present invention thus relates, in one aspect, to a pharmaceutical composition comprising a pharmaceutically acceptable carrier and at least one CCR5 antagonist, for reducing liver inflammation and liver damage caused by HCV infection.

In another aspect, the present invention relates to the use of a CCR5 antagonist for the manufacture of a pharmaceutical composition for reducing liver inflammation and liver damage caused by HCV infection.

In a further aspect, the present invention relates to a method for treatment of a subject afflicted with HCV infection which comprises administering to said subject an amount of a CCR5 antagonist effective for reducing liver inflammation and liver damage caused by the HCV infection.

In a preferred embodiment of the invention, the CCR5 antagonist is administered at the early stages of the disease.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1A-1B show the trafficking of NK cells from the blood to the liver during Con A induced hepatitis. Ninety-six hours following Con A intravenous injection to 6-week-old C57BL/6 wild-type mice (n=18), the number of NK cells in the peripheral blood (PBL) rises (**Fig. 1A**) while their numbers in the liver is reduced significantly (**Fig. 1B**).

Fig. 2 shows the involvement of CCR5 in the trafficking of NK and T cells to the liver. NK and T cells trafficking to the liver were tested in mice deficient of CCR5 (CCR5KO) in comparison with control, wild-type mice (CTRL), following Con A intravenous injection.

DETAILED DESCRIPTION OF THE INVENTION

T lymphocytes infiltrating the liver of HCV infected patients express the chemokine receptor CCR5. The ligands for CCR5, MIP-1α and MIP-1β, were shown to be expressed by vessels within the portal tracts. Thus, the chemokine receptor CCR5 may play an important role in the recruitment of immune cells to the liver during HCV infection and a CCR5 antagonist may be useful to treat liver damage caused by HCV infection.

The present invention is directed to the role of CCR5 in modulating hepatic inflammation, fibrosis and liver damage.

The present invention thus provides, in one aspect, a pharmaceutical composition comprising a pharmaceutically acceptable carrier and at least one CCR5 antagonist for reducing liver inflammation and liver damage caused by HCV infection.

In a preferred embodiment, the CCR5 antagonist is administered at early stages of the disease, before fibrosis is noted in patients where liver damage can be detected by testing for the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), or when fibrosis is noted but before it has developed into cirrhosis.

HCV causes at least 80% of posttransfusion hepatitis cases and a substantial proportion of sporadic acute hepatitis cases. HCV is a major cause of chronic hepatitis (about 75% of cases of hepatitis C become chronic) and consequent liver fibrosis and cirrhosis. HCV may also act synergistically to exacerbate alcohol-induced cirrhosis and liver damage, and vice-versa. Hepatocellular carcinoma is a risk in HCV-induced cirrhosis.

Many chronic hepatitis C patients are asymptomatic. In laboratory tests, the levels of the aminotransferases AST and ALT are elevated in HCV patients at the chronic stage of the disease.

Fibrosis is a common response to hepatocellular necrosis or injury, which may be induced by a wide variety of agents, including viral infections of the liver. The development of fibrosis from active deposition of collagen is a consequence of liver cell injury, particularly necrosis, and inflammatory cells. Cirrhosis is the end stage of many forms of liver injury characterized initially by fibrosis. The progression of fibrosis to cirrhosis depends on the extent of injury, the presence of continuing damage, and the response of the liver to damage.

The CCR5 chemokine receptor has been identified as co-receptor for HIV-1 entry in cells. Therefore, CCR5 antagonists that interfere with the interaction between the CCR5 receptor and HIV-1 can block HIV-1 entry into the cell.

Since HIV-1 needs to attach to CCR5 in order to infect the cells, several CCR5 antagonists have been proposed as HIV-1 inhibitors. These CCR5 antagonists include antibodies, modified chemokines and fractions thereof, peptides derived from said chemokines, and small organic molecules.

In accordance with the present invention, it is now proposed to use the CCR5 antagonists for treatment of HCV infections in order to reduce liver inflammation and liver damage caused by the HCV infection.

According to the present invention, the CCR5 antagonist is selected from anti-CCR5 antibodies, modified chemokines, preferably chemokines that are CCR5 ligands such as MIP-1α, MIP-1β and RANTES, and fractions thereof, peptides derived from said chemokines, and small organic molecules.

According to one embodiment of the invention, the CCR5 antagonist is an anti-CCR5 antibody, preferably a monoclonal antibody or an antigen-binding fragment thereof. The anti-CCR5 monoclonal antibody or antigen-binding fragment thereof inhibits binding of one or more chemokines selected from the group consisting of MIP-1α, MIP-1β and RANTES to the CCR5 receptor, and/or inhibits one or more functions associated with binding of said one or more chemokines to

the CCR5 receptor. In one preferred embodiment, the monoclonal antibody is humanized.

Without limiting the scope of the anti-CCR5 monoclonal antibodies that can be used according to the invention, examples are the anti-CCR5 antibodies described in US Patent No. 6,528,625, hereby incorporated by reference in its entirety as if fully disclosed herein. Specific examples are PRO 140, a monoclonal antibody specific to CCR5 (Trkola et al., 2001) and PRO 367, both in preclinical phase, and PRO 542, in Phase II clinical trials for treatment of HIV infections (Progenics Pharmaceuticals Inc.).

According to another embodiment of the invention, the CCR5 antagonist is a modified chemokine or a fraction thereof. In one preferred embodiment, the chemokine is RANTES, and the modification is at the N-terminal of the molecule. Without limiting the scope of the modified chemokines or fractions thereof that can be used according to the invention, examples are the N-terminal modifications of RANTES described in US Patent No. 6,168,784, hereby incorporated by reference in its entirety as if fully disclosed herein. Specific examples are N-terminal modifications of RANTES resulting in antagonists that can block HIV-1 infection without signaling calcium flux (Mack et al., 1998; Proudfoot et al., 1996). These modifications include N-terminal truncation [RANTES 9-68, RANTES 3-68] (Arenzana-Seisdedos et al., 1996), and addition of methionine ("Met-RANTES") or aminooxypentane ("AOP-RANTES") at the N-terminus of RANTES (Mack et al., 1998; Simmons, et al., 1997). It has been reported that the Met-RANTES and AOP-RANTES derivatives are antagonists of RANTES. Further, N-terminally modified RANTES, with a higher affinity for CCR5 than native RANTES are more potent than native RANTES in blocking infection (Simmons, et al., 1997).

According to a further embodiment of the invention, the CCR5 antagonist is a peptide derived from a chemokine. In one preferred embodiment, the chemokine is RANTES, and the peptides are based on a decapeptide corresponding to positions 1-10 of RANTES such as the peptides [Ac-(10Ala-RANTES 1-10)-NH2] andAc-(10Ala, 11Ala-RANTES 5-14)-NH2 (Nishiyama et al., 1997).

According to a still further embodiment of the invention, the CCR5 antagonist is a small-molecule CCR5 antagonist. There is ongoing in the art a substantial investigation of different classes of modulators of chemokine receptor activity, especially that of the CCR5 chemokine receptor. It is desired that the invention will encompass all such small-molecule CCR5 antagonists already described or to be discovered in the art such as, but not limited to, the compounds described in the following patents: the anilide derivatives disclosed in US 6,235,771, the piperidine derivatives disclosed in US 6,387,930, the piperazine derivatives disclosed in US 6,391,865, the tetramic acid-type compounds disclosed in US 6,476,062, the pyrrolidine derivatives disclosed in US 6,531,484, the cyclic amine compounds disclosed in US 6,562,978, and the compounds disclosed in US 6,586,430. Further disclosures of interest include: WO 00/39125, WO 00/38680, WO 98/30218, WO 98/25617, WO 98/025605, WO 98/025604, WO 98/002151, WO 98/004554, and WO 97/024325. All these patents and patent applications are hereby incorporated by reference in their entirety as if fully disclosed herein.

According to preferred embodiments of the invention, the CCR5 antagonist is selected from the following compounds currently in Phase I or Phase II clinical trial, as shown in Table 6 hereinafter: the orally bioavailable molecules TAK-220, SCH-C (SCH 351125) (Strizki et al., 2001), SCH-D (SCH 417690) (Tagat et al., 2004), AK-602, and UK-427,857.

Any other CCR5 antagonist for use in the present invention can be identified by methodology known in the art, such as the assay for CCR5 binding following procedures disclosed by Combadiere et al. (1996). In particular, the compounds should be capable of preventing binding of all known chemokine ligands to CCR5 in such binding assays.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be acceptable in the sense that it is compatible with the other ingredients of the composition and are not deleterious to the recipient thereof.

The term "carrier" refers to a diluent, adjuvant, excipient, or any other suitable vehicle. Such pharmaceutical carriers can be sterile liquids such as water and oils.

The pharmaceutical composition of the invention can be administered systemically, for example by parenteral, *e.g.* intravenous, intraperitoneal or intramuscular injection, or by any other suitable route including subcutaneous, transcutaneous, topical, intraarticular, subconjunctival, or mucosal, *e.g.* oral, intranasal, or intraocular. The composition will be formulated according to the type of CCR5 antagonist chosen, may it be an antibody, a modified chemokine, a peptide or a small moelecule. These preparations can be prepared by conventional methods known to those skilled in the art, for example as described in Remington: The Science and Practice of Pharmacy, A.R. Gennaro, ed., 20th edition, 2000.

For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents; fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats);

emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., ationd oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound. For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner. For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetra-fluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compositions of the invention can also be delivered in a vesicle, in particular in liposomes. In another embodiment, the compositions can be delivered in a controlled release system.

For the treatment of HCV, a protocol of administration may be adopted that is similar to the protocol proposed for the same CCR5 antagonist in the treatment of HIV.

The invention will now be illustrated by the following non-limiting Examples.

EXAMPLES

Materials and Methods

(i) Subjects - A total of 250 Jewish Israeli liver patients who had been followed-up at the Liver and Gastroenterology Units, Department of Medicine, Hadassah University Hospital, Ein-Karem, Jerusalem, Israel, and at the Liver Institute, Rabin Medical Center, Petach Tikva, Israel, agreed to participate in the study. One hundred and twenty seven (127) were anti-HCV positive chronic liver disease patients, 48 were anti-HCV positive who had undergone liver transplantation due to end-stage liver cirrhosis, and 75 were anti-HCV negative and had undergone liver transplantation due to non-HCV associated liver diseases. For each HCV seropositive patient, the first liver biopsy performed following disease at diagnosis was assessed blindly by a hepatopathologist and scored according to the Knodell scoring system (Knodell et al., 1981). Informed consent was obtained from all participants according to the Guidelines of the Institutional Ethics Committee.

Liver injury was tested in these patients by measuring the alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB) and bilirubin (BIL) levels.

(ii) Genotyping Methods - Genomic DNA was extracted from 100 µl peripheral blood with chemagic kit (chemagen AG, Baesweiler, Germany) according to manufacturer's instructions. PCR for the detection of CCR5Δ32 genotype was done as described by Wu et al. (1997). Shortly, upstream and downstream oligonucleotide primers for amplifying the CCR5 gene, corresponding to the second extracellular region of CCR5, were used. The wild-type CCR5 allele gave rise to a PCR fragment of 174 bp, whereas the allele with the deletion gave rise to a 142 bp fragment. For each PCR reaction (100 µl volume), 1 µg genomic DNA was first denatured at 95°C for 5 min, and amplified by 5 cycles of PCR (94°C, 45 s; 55°C, 45 s; 72°C, 45 s) followed by an additional 35 cycles (94°C, 45 s; 62°C,

45 s; 72°C, 30 s). The reaction products (25 µl) were run on a 4% NuSieve® GTG® agarose gel (Cambrex) and DNA bands were stained by ethidium bromide.

(iii) Statistical analysis

(a) Inflammation - With regard to liver inflammation, four parameters of disease activity were assessed: A – interface hepatitis (periportal); B –portal inflammation; C – lobular necrosis; and D – liver fibrosis (Knodell et al., 1981). Among 63 cases with data on the inflammatory parameters (A,B,C) and fibrosis (D), 47 were of the phenotype ++, and 16 of the phenotype +-. The distribution of the degree of inflammation was examined, and Kolmogorov-Smirnov's test for normality with Lilliefors significance correction was applied within each phenotype group. Consequently, the non-parametric Mann-Whitney test was used in the comparisons between the phenotype groups. Analyses were performed for (i) total sample, and (ii) within subgroups defined by the level of fibrosis (low, high). These two analyses were repeated for Ashkenazi subjects (AJI).

(b) Liver function - Liver histological sections of biopsies were performed following disease detection, blindly evaluated by a histopathologist and compared with liver function tests collected at the same time. The distribution of liver functions tests performed at the time of first liver biopsy was examined, and Kolmogorov-Smirnov's test for normality with Lilliefors significance correction was applied within each phenotype group. Consequently, the non-parametric Mann-Whitney test was used in the comparisons. Analyses were performed for (i) total sample, and (ii) within subgroups defined by the level of fibrosis (low, high). Those two analyses were repeated for Ashkenazi subjects (AJI).

Example 1. CCR5Δ32 allele frequency and susceptibility to HCV infection

The frequency of CCR5Δ32 allele among Jewish Israeli population is about 10 %. Israelis of Ashkenazi ancestry have the highest frequency, 10-13.8%, while only 2.08-4.8% of Israelis of Sephardi ancestry are carriers of the mutation. Another report has found that, among Ashkenazi Jews, the frequency of CCR5Δ32 allele is 20.93% (Maayan et al., 2000; Kantor et al., 1999).

The first issue we addressed was the susceptibility of CCR5Δ32 homo/heterozygotes to infection with HCV. The frequency of the CCR5Δ32 allele was assessed in 175 mixed Jewish Israeli **(JI)** and Ashkenazi Jewish Israelis **(AJI)** HCV patients. In agreement with the publication by Promrat et al. (2003), we have not found an association between HCV infection and the prevalence of CCR5Δ32 allele. Only one of the 175 HCV seropositive participants in the study was homozygote for this allele (0.57%). The prevalence of CCR5Δ32 allele among HCV patients was 16.5%. Considering the fact that most of HCV patients in our study are of Ashkenazi origin (72.5%), and since the prevalence of CCR5Δ32 allele in healthy Ashkenazi Jewish Israelis is between 10-20%, we suggest that there is no adverse host effect for this mutation with regard to HCV infection (Maayan et al., 2000; Kantor et al., 1999). In addition, we found that the frequency of CCR5Δ32 allele is nearly similar among HCV patients, HCV patients who had undergone liver transplantation and liver transplanted patients that are HCV seronegative (18%). This may suggest that CCR5Δ32 allele does not contribute to the acceleration of disease towards end-stage liver disease.

Example 2. CCR5Δ32 heterozygosity is associated with reduced liver inflammation

Considering the well documented role of CCR5 in recruitment of immune cells to the portal tracts of HCV inflamed livers, we decided to further examine the inflammatory process in the liver of CCR5Δ32 heterozygotes compared to individuals with normal phenotype (Shields et al., 1999; Goddard et al., 2001). To this end, the first liver biopsy performed following disease diagnosis and patient specific clinical data parameters at the same time were collected. A total of 16 biopsies from CCR5Δ32 heterozygotes and 47 biopsies from control HCV patients were examined; demographic characteristics for these patients are presented in Table 1A. No significant differences between the two groups were found with regard to response to anti-HCV treatment, HCV-associated autoimmune diseases,

gender and age. Among the group of patients for whom demographic and clinical parameters were fully collected (n=68 → 21 CCR5Δ32 heterozygotes + 47 control HCV patients) the most common cause of infection was blood transfusion 18/68 (26.4%), 3/68 (4.4%) were IV drug addicts and 3/68 (4.4%) were infected when working with contaminated blood samples. No data regarding cause of infection of other study participants was available 44/68 (64.7%). Interestingly, whereas the susceptibility of HCV patients carrying the CCR5Δ32 allele to HCV 1B strain was similar, these patients were not infected with the HCV 3A strain (Table 1B). This may suggest a role for CCR5 or immune cells that are dependent for their trafficking to liver on CCR5, in the clearance of this strain. HCV 3A is a hepatitis virus strain that better responds to IFN-α therapy than other HCV strains (McKechnie et al., 2000). IFN-α induces the production of CCL3, a CCR5 ligand in the liver (Salazar-Mather et al., 2002). It is possible then that CCR5 is involved in a cascade of events or recruitment of immune cells that negatively regulates the production of IFN-α in the liver.

Table 1. Demographic characteristics of study participants

	Phenotype	N	Age (mean)	Std. Err	t-test	Gender
A- JI	++	46	42.87	1.87		M(37), F(9)
	+-	16	48.44	3.86	**0.158**	M(12), F(4)
B- AJI	++	30	39.93	2.27		M(25), F(5)
(45/62= 72.5%)	+-	15	47.67	4.04	**0.078**	M(11), F(4)

JI – Jewish Israeli; AJI – Ashkenazi Jewish Israeli

Further assessment of the clinical outcomes of HCV infection were performed on patients that had undergone a liver biopsy, which was graded using the Knodell score. With regard to liver inflammation, four parameters of disease activity were assessed: A – interface hepatitis (periportal); B –portal inflammation; C – lobular necrosis; and D – liver fibrosis (Knodell et al., 1981). A histophatological analysis of liver biopsy showed a significant (A+B+C $p<0.015$) decrease in the level of liver inflammation in HCV Jewish Israeli (JI) patients

heterozygotes for CCR5Δ32 allele (Table 2A) relative to control. In each of the inflammatory parameters, A, B, C, a significant reduction ($p<0.019$, 0.037, 0.034 respectively) in inflammatory activity was observed (Table 2A). Liver fibrosis was not significantly different between the two groups ($P=0.98$) (Table 2A).

Similar results were obtained when a total of 15 biopsies from CCR5Δ32 heterozygotes and 31 biopsies from control HCV Ashkenazi Jewish Israelis (AJI) patients were examined. A histophatological analysis of liver biopsy showed a significant (A+B+C $p<0.006$) decrease in the level of liver inflammation in HCV AJI patients heterozygotes for CCR5Δ32 (Table 2B) relative to control. In each of the inflammatory parameters A, B, C, a reduction ($p<0.003$, 0.074, 0.025 respectively) in inflammatory activity was observed. Liver fibrosis was not significantly different between the two groups ($p<0.54$) (Table 2B).

From the observation that CCR5Δ32 heterozygotes showed only partial reduction in expression of CCR5, but not a total loss of receptor expression and function, it is tempting to speculate that a total blockage of CCR5 in HCV patients may have an even more profound effect in reducing the inflammatory process in the liver (Venkatesan et al., 2002). Indeed, the only patient in our study homozygote for the CCR5Δ32 allele had a very low index of inflammation (score 2) and almost no progression of disease over time.

HCV-associated liver inflammation is a complex process involving multiple cytokines and chemokines (Heydtmann et al., 2001). In the early phase following HCV infection, the INF-γ induced chemokines CXCL9, CXCL10 and their receptor CXCR3, are upregulated in the liver (Arai et al., 2002; Dufour et al., 2002; Narumi et al., 1997). Furthermore, CCR5 positive cells have been shown to accumulate in the portal tracts where the chemokine CCL3 and CCL4 are expressed (Shields et al., 1999; Apolinario et al., 2002). Later on, CCL21 and CXCL12, traditionally considered as homeostatic chemokines, are upregulated and may participate in shaping the chronic inflammatory response. Indeed, it has been shown that CCL21 and CXCL12 support the recruitment of immune cells to chronically inflamed livers and that liver infiltrating lymphocytes express the chemokine receptors CCR7 and

CXCR4 (Terada et al., 2003; Grant et al., 2002; Goddard et al., 2001). Our results suggest that although liver inflammation is mediated by multiple cytokines and chemokines, it may be modulated at least periodically by blocking the interaction of a specific chemokine receptor with its ligands.

Table 2. Inflammatory activity in CCR5Δ32 heterozygotes and control

	Phenotype		A+B+C	A+B+C+D	A	B	C	D
A- ЛІ	++	**Mean**	**6.57**	**8.23**	**1.98**	**2.57**	**2.02**	**1.66**
		N	47	47	47	47	47	47
		Std. Err	0.33	0.43	0.17	0.11	0.14	0.15
	+-	**Mean**	**4.56**	**6.28**	**1.19**	**2**	**1.38**	**1.72**
		N	16	16	16	16	16	16
		Std. Err	0.77	1.05	0.37	0.27	0.27	0.36
		statistic test						
		Mann-Whitney U	224.5		237	252	252	375
		Asymp.Sig.(2-tailed)	**0.015**		**0.019**	**0.037**	**0.034**	**0.986**
B- АЛІ	++	**Mean**	**6.65**	**8.35**	**2.13**	**2.48**	**2.03**	**1.71**
		N	31	31	31	31	31	31
		Std. Err	0.43	0.52	0.21	0.15	0.19	0.17
	+-	**Mean**	**4.2**	**5.8**	**1**	**1.93**	**1.27**	**1.6**
		N	15	15	15	15	15	15
		Std. Err	0.73	0.99	0.34	0.27	0.27	0.36
		statistic test						
		Mann-Whitney U	116.5		114	168	145	209
		Asymp.Sig. (2tailed)	**0.006**		**0.003**	**0.074**	**0.025**	**0.54**

ЛІ – Jewish Israeli; AJI – Ashkenazi Jewish Israeli
A – interface hepatitis (periportal); B – portal inflammation; C – lobular necrosis; D – liver fibrosis

Example 3. Reduced CCR5Δ32-associated liver inflammation is observed in early stages of liver disease

In order to further characterize the phenomenon of CCR5Δ32-associated reduction in liver inflammation, we next assessed, in a patient specific manner, the histopathological scoring. CCR5Δ32 heterozygotes and patients with normal CCR5 phenotype were further divided according to the level of liver fibrosis, assuming that patients with low fibrosis scoring were in earlier stages of disease.

In both CCR5Δ32 heterozygotes and controls from JI and AJI, we found that patients with low fibrosis had lower inflammatory activity: score 3.0 vs. 7.17 for CCR5Δ32 heterozygotes and 5.66 vs. 8.06 for control group, respectively, in JI; and score 3.0 vs. 6.6 for CCR5Δ32 heterozygotes and 5.89 vs. 7.83 for control group, respectively, in AJI (Tables 3A, 3B).

Moreover, the effects of CCR5Δ32 allele on liver inflammation are shown to be highly significant in JI and AJI patients with low fibrosis: score 3.00 vs 5.65 for CCR5Δ32 heterozygotes vs. control group, respectively, in JI ($p=0.001$); and score 3.0 vs. 5.89 for CCR5Δ32 heterozygotes vs. control group, respectively, in AJI ($p=0.002$) (Tables 3A, 3B). There was no effect of the CCR5Δ32 allele on liver inflammation in JI and AJI patients with high fibrosis (Tables 3A, 3B).

Taken together, these data suggest that CCR5Δ32 allele is associated with reduced liver inflammation in early stages of HCV-associated liver disease. In accordance with the well-documented expression of CCR5 ligands in the portal tracts, we found a profound effect of CCR5Δ32 allele on the levels of interface hepatitis (parameter A) and portal inflammation (parameter B) (Grant et al., 2002; Goddard et al., 2001). As HCV-associated liver inflammation progresses and fibrosis develops, additional chemokines such as CCL21 and CXCL12 are upregulated, contributing to the establishment of neolymphoid active follicles in portal tracts and fibrotic septi (Terada et al., 2003; Grant et al., 2002; Goddard et al., 2001; Boisvert et al., 2003). Correspondingly, we have found that as disease progresses and fibrosis develops, the effect of CCR5Δ32 on the inflammatory response was reduced.

Table 3. Inflammatory activity in CCR5Δ32 heterozygotes and control according to level of fibrosis

	Fibrosis	Phenotype		A+B+C	A	B	C
A- JI	Low	++	Mean	**5.66**	**1.45**	**2.52**	**1.69**
			N	29	29	29	29
			Std. Err	0.39	0.19	0.15	0.17
		+-	Mean	3	0.3	1.6	1.1
			N	10	10	10	10
			Std. Err	0.56	0.15	0.31	0.28
	High	++	Mean	**8.06**	**2.83**	**2.67**	**2.56**
			N	18	18	18	18
			Std. Err	0.41	0.22	0.18	0.2
		+-	Mean	7.17	2.67	2.67	1.83
			N	6	6	6	6
			Std. Err	1.3	0.56	0.33	0.54
	statistic test						
	Low		Mann-Whitney U	48.5	46.5	74.5	95.5
			Exact Sig. (2-tailed)	**0.001**	**0.001**	**0.022**	**0.112**
	High		Mann-Whitney U	50	54	54	37
			Exact Sig. (2-tailed)	**0.82**	**1**	**1**	**0.28**
B- AJI	Low	++	Mean	**5.89**	**1.79**	**2.47**	**1.63**
			N	19	19	19	19
			Std. Err	0.55	0.25	0.19	0.23
		+-	Mean	**3**	**0.3**	**1.6**	**1.1**
			N	10	10	10	10
			Std. Err	0.56	0.15	0.31	0.28
	High	++	Mean	**7.83**	**2.67**	**2.5**	**2.67**
			N	12	12	12	12
			Std. Err	0.56	0.31	0.26	0.22
		+-	Mean	**6.6**	**2.4**	**2.6**	**1.6**
			N	5	5	5	5
			Std. Err	1.44	0.6	0.4	0.6
	statistic test						
	Low		Mann-Whitney U	30.5	21.5	51.5	68.5
			Exact Sig. (2-tailed)	**0.002**	**0.0003**	**0.045**	**0.228**
	High		Mann-Whitney U	23.5	27	28.5	16
			Exact Sig. (2-tailed)	**0.506**	**0.799**	**0.879**	**0.16**

JI – Jewish Israeli; AJI – Ashkenazi Jewish Israeli

A – interface hepatitis (periportal); B – portal inflammation; C – lobular necrosis

Example 4. Reduced CCR5Δ32-associated liver inflammation is coupled with reduced liver damage

In order to further characterize the association between liver inflammation and liver damage, we measured the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB) and bilirubin (BIL) in HCV patients participating in this study. We found no significant differences between CCR5Δ32 heterozygotes and control group in both JI and AJI (Tables 4A, 4B).

Table 4. Liver injury parameters in CCR5Δ32 heterozygotes and control

	Phenotype		AST	ALT	ALB	BIL
A- JI	++	**Mean**	**100.59**	**165.22**	**41.49**	**12.87**
		N	37	37	37	36
		Std. Err	23.56	47.16	0.46	1.07
	+-	**Mean**	**98.53**	**119.53**	**41.41**	**14.43**
		N	15	15	16	16
		Std. Err	24.43	32.25	0.44	2.22
	statistic test					
		Mann-Whitney U	249.5	239	280	265
		Asymp. Sig. (2-tailed)	**0.57**	**0.44**	**0.75**	**0.65**
B- AJI	++	**Mean**	**107.41**	**188.3**	**41.63**	**12.23**
		N	27	27	27	27
		Std. Err	31.87	64.26	0.54	1.14
	+-	**Mean**	**80.71**	**95.21**	**41.17**	**13.85**
		N	14	14	15	15
		Std. Err	17.96	22.75	0.4	2.29
	statistic test					
		Mann-Whitney U	155.5	147	175.5	185.5
		Asymp. Sig. (2-tailed)	**0.36**	**0.25**	**0.47**	**0.65**

JI – Jewish Israeli; AJI – Ashkenazi Jewish Israeli
AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALB – albumin; BIL – bilirubin

However, when patients were further divided according to the level of fibrosis, we found lower ALT (p=0.053, 0.058) and AST (p=0.017, 0.021) levels, but not ALB (p=0.451, 0.675) and BIL (p=0.923, 0.902) levels, in both JI and AJI, respectively (Tables 5A, 5B). These findings suggest that in early stages of disease, before development of fibrosis, reduced CCR5Δ32-associated liver inflammation is

coupled with reduced liver damage. Interestingly, CCR5Δ32 heterozygotes are older [mean=48.8 (JI), 47.7 years (AJI)] relative to control patients [mean= 42.87 (JI, p=0.156), 39.93 (AJI, p=0.078)] years (Table 1). Since the date of infection in most HCV cases is uncertain, it is hard to determine whether this age difference represents a delayed disease progression. Interestingly, the sole CCR5Δ32 homozygote patient in our study had a very slow progressing disease.

Table 5. Liver injury parameters in CCR5Δ32 heterozygotes and control according to level of fibrosis

	Fibrosis	Phenotype		AST	ALT	ALB	BIL
A- JI	Low	++	Mean	**110.48**	**191.83**	**42**	**12.1**
			N	23	23	23	23
			Std. Err	37.63	75.3	0.51	1.28
		+-	Mean	**44.22**	**61.33**	**41.35**	**13.4**
			N	9	9	10	10
			Std. Err	10.05	19.28	0.53	3.27
	High	++	Mean	**84.36**	**121.5**	**40.64**	**14.23**
			N	14	14	14	13
			Std. Err	9.55	16.74	0.86	1.91
		+-	Mean	**180**	**206.83**	**41.5**	**16.13**
			N	6	6	6	6
			Std. Err	41.52	62.03	0.85	2.53
	statistic test						
	Low		Mann-Whitney U	47	57.5	95.5	112
			(2 tailed)	**0.017**	**0.053**	**0.451**	**0.923**
	High		Mann-Whitney U	14.5	29	37.5	29.5
			(2 tailed)	0.02	0.312	0.718	0.416
B- AJI	Low	++	Mean	**122.41**	**226.12**	**41.82**	**11.01**
			N	17	17	17	17
			Std. Err	50.26	101.2	0.65	1.14
		+-	Mean	44.22	61.33	41.35	13.4
			N	9	9	10	10
			Std. Err	10.05	19.28	0.53	3.27
	High	++	Mean	**81.9**	**124**	**41.3**	**14.3**
			N	10	10	10	10
			Std. Err	13.18	23.06	0.99	2.35
		+-	Mean	**146.4**	**156.2**	**40.8**	**14.76**
			N	5	5	5	5
			Std. Err	29.88	43.9	0.58	2.61
	statistic test						

	Low		**Mann-Whitney U**	34	41.5	76.5	82.5
			(2 tailed)	**0.021**	**0.058**	**0.675**	**0.902**
	High		**Mann-Whitney U**	10.5	21	19.5	21
			(2 tailed)	**0.075**	**0.679**	**0.513**	**0.679**

JI – Jewish Israeli; AJI – Ashkenazi Jewish Israeli
AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALB – albumin; BIL – bilirubin

Example 5. CCR5 regulates the trafficking of NK cells to the liver during ConA-induced hepatitis.

Our results suggest that CCR5 is involved in a cascade of events or recruitment of immune cells that negatively regulates the production of IFN-α in the liver. The lectin Con A induces immune cell-mediated hepatitis (Schumann et al., 2000, Takeda et al., 2000). Con A-induced hepatitis (Con A-hepatitis) is a hepatitis model in which hepatic injury is supposed to be caused by cytokines (TNF-α and IFN-γ)from activated T cells.

Following intravenous injection of Con A, NK cells are mobilized from the bone marrow (BM) and spleen into the blood and subsequently recruit into the liver. In the peripheral blood, the numbers of NK cells increase initially, while at 24 hr their numbers decrease concomitant with the massive efflux of NK cells to the liver. Ninety-six hr following Con A injection to wild type, C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME, USA, n=18), the number of NK cells in the peripheral blood rises while their numbers in the liver is reduced significantly (**Figs. 1A, B**). Following Con A injection, the cytokines TNF-α and IFN-γ are dramatically up-regulated and are shown to be critical for the formation of liver damage and inflammation. In addition to cytokines, the CCR5 chemokine receptor ligand, CCL3, is shown to play a role in the recruitment of NK cells to the inflamed liver (Biron and Brossay, 2001). Considering the role of CCL3 in the establishment of Con A-induced liver damage and the suggested role of the CCR5 in the recruitment of NK cells to the liver, we analyzed whether NK cells trafficking to the liver would be affected in mice deficient of CCR5 (C57BL/6-CCR5-KO, Jackson Laboratory, Bar Harbor, ME, USA, n=6) in comparison with C57BL/6 wild-type mice (n=6).

Following Con A intravenous injection, the recruitment of NK cells in the liver was reduced significantly in CCR5 deficient mice (**Fig. 2**). Moreover, the number of T cells accumulating in the liver of mice deficient of CCR5 increased by 5 folds (**Fig. 2**). These results suggest that the recruitment to the liver of NK cells negatively regulates the entry of T cells and helps in restraining the inflammatory response

Example 6. Protocol for human studies of CCR5 antagonists in reducing liver inflammation and liver damage in HCV infections

The lack of a small animal model for hepatitis C requires that clinical studies be carried out in human patients.

The patients to be enrolled in the studies are selected from (HCV+) patients in chronic stage of the disease (non-acute), patients that are resistant to IFN-α and ribavirin therapy, and patients that have high levels of AST and ALT in their blood. The CCR5 antagonist, for example those shown in Table 6, is administered in a protocol similar to the protocol for treatment of HIV infection . During therapy, AST and ALT levels in the patients' blood are tested. Liver biopsy is performed both prior to and following therapy.

Table 6. CCR5 antagonists

Compound	Class of Compound	Phase of Development	Pharmaceutical Co.
TAK 220	CCR5 antagonist	Phase I/II	Takeda
PRO 542	CCR5 antagonist	Phase II	Progenics
AK 602	CCR5 antagonist	Phase I	Ono/Moravek
SCH D	CCR5 antagonist	Phase I /II	Schering Plough
PRO 140	CCR5 antagonist	Preclinical	Progenics
SCH C	CCR5 antagonist	Phase I/II	Schering Plough
UK-427,857	CCR5 antagonist	Phase II	Pfizer

Discussion

The results above show that inflammatory activity as well as serum levels of hepatocytes cytosolic enzymes (ALT and AST), in early stages of hepatitis C disease, before development of fibrosis, were significantly reduced in patients carrying the CCR5Δ32 allele relative to control. Further, in agreement with Promrat et al., 2003, we have not found an increased prevalence of CCR5Δ32 homozygotes among HCV patients.

We also found that liver inflammatory activity in early stages of disease, before development of fibrosis, was significantly reduced in JI (P=0.001) and AJI (p=0.002) patients carrying the CCR5Δ32 allele relative to patients with normal genotype. Complimentary, lower ALT (p=0.053) and AST (P=0.017) levels, but not ALB (P=0.451) and BIL (P=0.923) levels, were detected in the blood of HCV patients heterozygotes for CCR5Δ32 allele control group. The frequency of CCR5Δ32 allele among HCV patients is 16.5% (29/175) while the frequency of this allele in Israeli population is between 10-20%. Only one HCV patient was homozygote for CCR5Δ32 allele (0.57%).

These observations indicate that CCR5Δ32 allele does not alter the susceptibility to HCV infection. However, we suggest that decreased levels of CCR5 reduce liver inflammation and damage in early stages of disease. These findings thus mark CCR5 as a target for novel immune modulating drugs for the treatment of HCV.

These findings indicate that the CCR5Δ32 allele has no adverse host effect with respect to susceptibility to HCV infection or accelerated disease progression. However, reduced liver inflammation and liver damage in early stages of disease are associated with the CCR5Δ32 allele in Jewish Israeli (JI) and Ashkenazi Jewish Israelis (AJI). Further multi-center studies can better characterize the effects of CCR5Δ32 allele on HCV disease progression and the possibility to delay disease course by novel immune modulating drugs targeting CCR5.

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CLAIMS:

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and at least one CCR5 antagonist for reducing liver inflammation and liver damage caused by HCV infection.

2. A pharmaceutical composition according to claim 1 for administration at the early stages of the disease.

3. A pharmaceutical composition according to claim 1 or 2, wherein said CCR5 antagonist is selected from an anti-CCR5 antibody, a modified chemokine or a fraction thereof, a peptide derived from a chemokine, and a small organic molecules.

4. A pharmaceutical composition according to claim 3, wherein said CCR5 antagonist is an anti-CCR5 antibody.

5. A pharmaceutical composition according to claim 4, wherein said anti-CCR5 antibody is a monoclonal antibody or an antigen-binding fragment thereof.

6. The pharmaceutical composition of claim 5, wherein said anti-CCR5 monoclonal antibody or antigen-binding fragment thereof inhibits binding of one or more chemokines selected from the group consisting of MIP-1α, MIP-1β and RANTES to the receptor.

7. The pharmaceutical composition of claim 6, wherein said antibody or antigen-binding fragment thereof inhibits one or more functions associated with binding of said one or more chemokines to the CCR5 receptor.

8. The pharmaceutical composition of claim 7, wherein said antibody is the anti-CCR5 monoclonal antibody designated PRO 542 or PRO 140.

9. A pharmaceutical composition according to claim 3, wherein said CCR5 antagonist is a modified chemokine, preferably a chemokine that is a CCR5 ligand.

10. A pharmaceutical composition according to claim 9 wherein said modified chemokine is a modified RANTES.

11. The pharmaceutical composition according to claim 10 wherein said modified RANTES is a N-terminal modified RANTES.

12. The pharmaceutical composition according to claim 11 wherein said N-terminal modification of RANTES is a compound selected from the group consisting of N-terminal truncation, addition of methionine and addition of aminooxypentyl to the N-terminus of the RANTES molecule.

13. The pharmaceutical composition according to claim 12 wherein said compound is RANTES 9-68, Met-RANTES, or AOP-RANTES.

14. The pharmaceutical composition according to claim 3, wherein said CCR5 antagonist is a peptide derived from a chemokine, wherein said chemokine is preferably a chemokine that is a CCR5 ligand.

15. The pharmaceutical composition according to claim 3, wherein said CCR5 antagonist is a small organic molecule.

16. The pharmaceutical composition according to claim 15, wherein said small organic molecule is TAK-220, SCH-C, SCH-D, AK-602, or UK-427,857.

17. The pharmaceutical composition according to any one of claims 1 to 16, for administration together with IFN-α therapy.

18. The pharmaceutical composition according to any one of claims 1 to 16, for administration together with combined IFN-α and ribavirin therapy.

19. Use of a CCR5 antagonist for the manufacture of a pharmaceutical composition for reducing liver inflammation and liver damage caused by HCV infection.

20. The use according to claim 19 for administration at the early stages of the disease.

21. The use according to claim 19 or 20, wherein said CCR5 antagonist is selected from an anti-CCR5 antibody, a modified chemokine or a fraction thereof, a peptide derived from a chemokine, and a small organic molecules.

22. The use according to claim 21, wherein said CCR5 antagonist is an anti-CCR5 antibody.

23. The use according to claim 22 wherein said anti-CCR5 antibody is a monoclonal antibody or an antigen-binding fragment thereof.

24. The use according to claim 23, wherein said anti-CCR5 monoclonal antibody or antigen-binding fragment thereof inhibits binding of one or more chemokines selected from the group consisting of MIP-1α, MIP-1β and RANTES to the receptor.

25. The use according to claim 24, wherein said antibody or antigen-binding fragment thereof inhibits one or more functions associated with binding of said one or more chemokines to the CCR5 receptor.

26. The use according to claim 25, wherein said antibody is the anti-CCR5 monoclonal antibody designated PRO 542 or PRO 140.

27. The use according to claim 21, wherein said CCR5 antagonist is a modified chemokine, preferably a chemokine that is a CCR5 ligand.

28. The use according to claim 27 wherein said modified chemokine is a modified RANTES.

29. The use according to claim 28 wherein said modified RANTES is a N-terminal modified RANTES.

30. The use according to claim 29 wherein said N-terminal modification of RANTES is a compound selected from the group consisting of N-terminal truncation, addition of methionine and addition of aminooxypentyl to the N-terminus of the RANTES molecule.

31. The use according to claim 30 wherein said compound is RANTES 9-68, Met-RANTES, or AOP-RANTES

32. The use according to claim 21, wherein said CCR5 antagonist is a peptide derived from a chemokine, wherein said chemokine is preferably a chemokine that is a CCR5 ligand.

33. The use according to claim 21, wherein said CCR5 antagonist is a small organic molecule.

34. The use according to claim 33, wherein said small organic molecule is TAK-220, SCH-C, SCH-D, AK-602, or UK-427,857.

35. The use according to any one of claims 19 to 34, for administration together with IFN-α therapy.

36. The use according to any one of claims 19 to 34, for administration together with combined IFN-α and ribavirin therapy.

37. A method for treatment of a subject afflicted with HCV infection which comprises administering to said subject an amount of a CCR5 antagonist effective for reducing liver inflammation and liver damage caused by the HCV infection.

38. The method according to claim 37 for treatment at the early stages of the disease.

39. The method according to claim 37, wherein said CCR5 antagonist is selected from the group consisting of an anti-CCR5 antibody, a modified chemokine or a fraction thereof, a peptide derived from a chemokine, and a small organic molecules.

40. The method according to claim 39, wherein said CCR5 antagonist is an anti-CCR5 antibody.

41. The method according to claim 40 wherein said anti-CCR5 antibody is a monoclonal antibody or an antigen-binding fragment thereof.

42. The method according to claim 41, wherein said anti-CCR5 monoclonal antibody or antigen-binding fragment thereof inhibits binding of one or more chemokines selected from the group consisting of MIP-1α, MIP-1β and RANTES to the receptor.

43. The method according to claim 42, wherein said antibody or antigen-binding fragment thereof inhibits one or more functions associated with binding of said one or more chemokines to the CCR5 receptor.

44. The method according to claim 43, wherein said antibody is the anti-CCR5 monoclonal antibody designated PRO 542 or PRO 140.

45. The method according to claim 39, wherein said CCR5 antagonist is a modified chemokine, preferably a chemokine that is a CCR5 ligand.

46. The method according to claim 45 wherein said modified chemokine is a modified RANTES.

47. The method according to claim 46 wherein said modified RANTES is a N-terminal modified RANTES.

48. The method according to claim 47 wherein said N-terminal modification of RANTES is a compound selected from the group consisting of N-terminal

truncation, addition of methionine and addition of aminooxypentyl to the N-terminus of the RANTES molecule.

49. The method according to claim 48 wherein said compound is RANTES 9-68, Met-RANTES, or AOP-RANTES

50. The method according to claim 39 wherein said CCR5 antagonist is a peptide derived from a chemokine, wherein said chemokine is preferably a chemokine that is a CCR5 ligand.

51. The method according to claim 39 wherein said CCR5 antagonist is a small molecule.

52. The method according to claim 51 wherein said small organic molecule is TAK-220, SCH-C, SCH-D, AK-602, or UK-427,857.

53. The method according to claim 37, wherein the treatment includes administration of IFN-α.

54. The method according to claim 37, wherein the treatment includes combined IFN-α and ribavirin therapy.

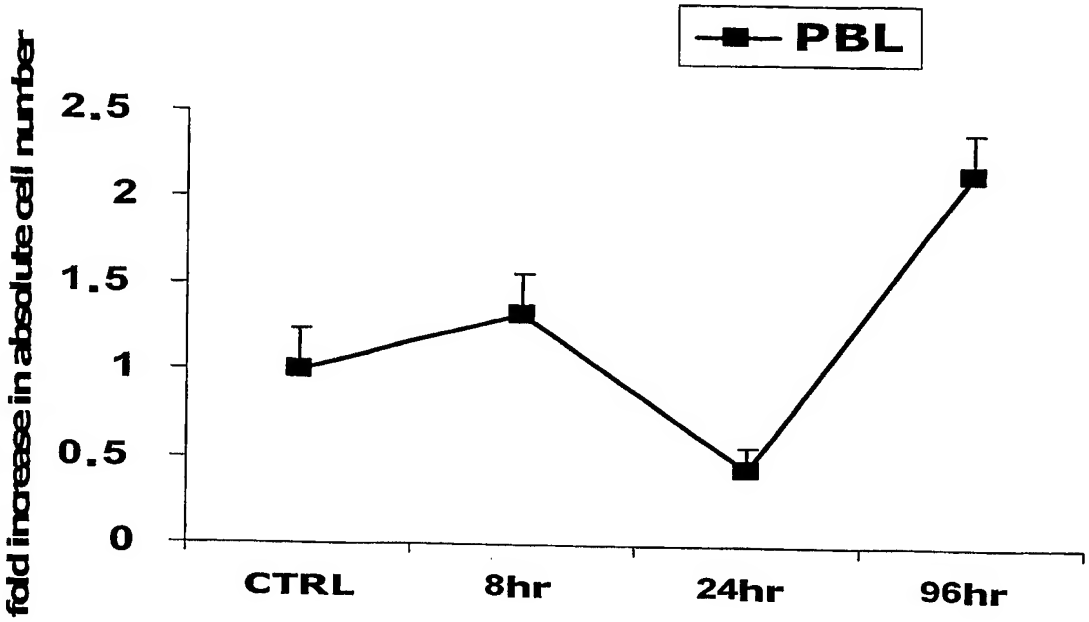
PBL
fold increase in absolute cell number
2.5
2
1.5
1
0.5
0
CTRL
8hr
24hr
96hr

Fig. 1A

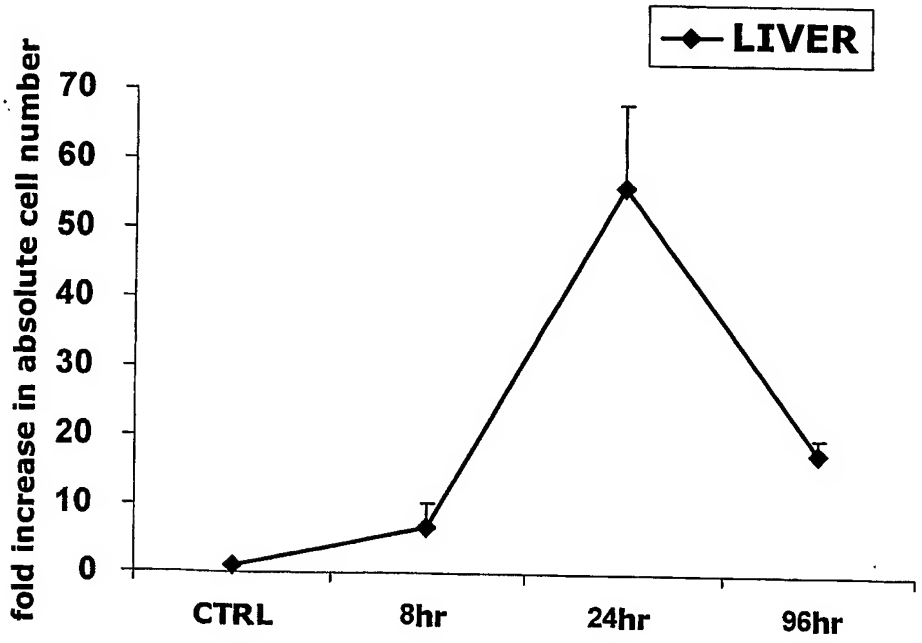
LIVER
fold increase in absolute cell number
70
60
50
40
30
20
10
0
CTRL
8hr
24hr
96hr

Fig. 1B

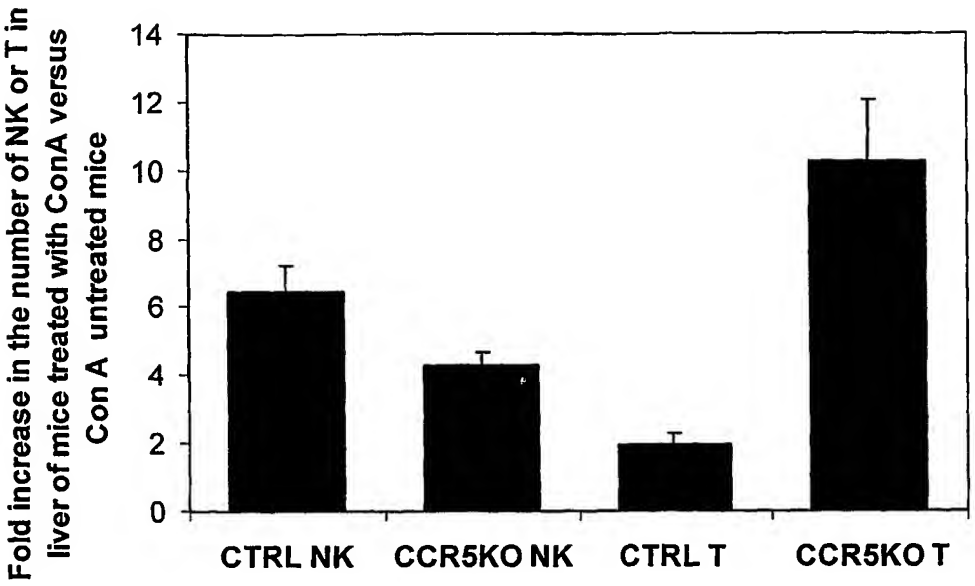
Fold increase in the number of NK or T in liver of mice treated with ConA versus Con A untreated mice
14
12
10
8
6
4
2
0
CTRL NK
CCR5KO NK
CTRL T
CCR5KO T

Fig. 2

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(57) Abstract: CCR5 antagonists such as anti-CCR5 antibodies, modified chemokines or a fraction thereof, peptides derived from such chemokines, and small organic molecules, are useful for reducing liver inflammation and liver damage caused by HCV infection.

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A. CLASSIFICATION OF SUBJECT MATTER
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 6,635,646 B1 (LAUGHLIN) 21 October 2003 (21.10.2003), see entire document.	1-3, 15-18 --- 4-14
Y --- A	US 2004/0023845 A1 (DORR et al) 05 February 2004 (05 February 2004), see entire document.	1-3 9-18 --- 1, 4-8
Y --- A	CILLERS et al., J Virol .Apr 2003 77(7):4449-4456. The CCR5 and CXCR4 Coreceptors Are Both Used by Human Immunodeficiency Virus Type 1 Primary Isolates from Subtype C,.see entire document..	1-16
A	ELSNER et al. Differential Activation of CC Chemokine Receptors by AOP-RANTES., J Biol Chem. 17 Mar 2000. 275(11)7787-7794.	1-18
A	IIZAWA et aL 10th Conference on Retroviruses and Opportunistic Infections. Boston, MA, USA. 10-14 February 2004. Abstract. "Anti-HIV-1 Activity of TAK-220, a small molecule CCR5 Antagonist"	1-3, 15-16

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